



The
Patent
Office

PCT/GB 99 / 04 377

09/868352

INVESTOR IN PEOPLE

GB99/4377

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 01 FEB 2000

WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Andrew Gurney

Dated

17th January 2000

THIS PAGE BLANK (USPTO)

Patents Form 1/77

Patents Act 1977
(Rule 16)



1/77
15 JAN 89 04:14:23-12 000590
21/07/77 0.0 - 50016 3

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

Request for the grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference

REP06017GB

2. Patent application number

(The Patent Office will fill in this part)

9901916.8

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Microscience Limited
67-68 Jermyn Street
London
SW1Y 6NY
United Kingdom

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

07304546001

4. Title of the invention

PROTEIN AND COMPOSITIONS CONTAINING IT

5. Name of your agent (if you have one)

GILL JENNINGS & EVERY

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number (if you know it)

745002 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

YES

- a) any applicant named in part 3 is not an inventor
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description

Claim(s)

Abstract

Drawing(s)

7

1

1

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. For the Applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Date

28 January 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

PERRY, Robert Edward
0171 377 1377

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

PROTEIN AND COMPOSITIONS CONTAINING IT

Field of the Invention

This invention relates to one protein, to vaccines
5 containing it, and to its use in therapy, for
immunisation.

Background to the Invention

Group B Streptococcus (GBS), also known as
Streptococcus agalactiae, is the causative agent of
10 various conditions. In particular, GBS causes:

Early onset neonatal infection.

This infection usually begins in utero and causes
severe septicaemia and pneumonia in infants, which is
lethal if untreated and even with treatment is associated
15 with a 10-20% mortality rate.

Late onset neonatal infection.

This infection occurs in the period shortly after
birth until about 3 months of age. It causes a
septicaemia, which is complicated by meningitis in 90% of
20 cases. Other focal infections also occur including
osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

Adult infections.

These appear to be increasingly common and occur
25 most commonly in women who have just delivered a baby,
the elderly and the immunocompromised. They are
characterised by septicaemia and focal infections
including osteomyelitis, septic arthritis, abscesses and
endophthalmitis.

30 *Urinary tract infections.*

GBS is a cause of urinary tract infections and in
pregnancy accounts for about 10% of all infections.

Veterinary infections.

GBS causes chronic mastitis in cows. This, in turn, leads to reduced milk production and is therefore of considerable economic importance.

5 GBS infections can be treated with antibiotics. However, immunisation is preferable. It is therefore desirable to develop an immunogen that could be used in a therapeutically-effective vaccine.

Summary of the Invention

10 According to the present invention, a partial GBS gene sequence, pho3-18, has been found which encodes a protein of unknown function.

 In one aspect of the invention, the use of this protein in a recombinant protein vaccine is described.
15 This vaccine may be administered to females either prior to or during pregnancy to protect mother and neonate against infection by GBS.

 The gene sequence may be first genetically altered to increase the antigenicity of the encoded protein.

20 Brief Description of the Drawings

 The invention will now be described in detail with reference to the accompanying figures, wherein:

 Figure 1 shows the nucleotide sequence of the insert of clone pho3-18 and the deduced amino acid sequence of
25 ORF3-18.

Description of the Invention

 Because of its extracellular or cell surface location, the protein of the present invention may be a suitable candidate for the production of therapeutically-
30 effective vaccines against GBS. The term "therapeutically-effective" is intended to include the prophylactic effect of the vaccines. For example, a recombinant protein may be used, as an antigen for direct administration to a patient. The protein may be isolated

directly from GBS expressed in any suitable expression system, e.g. *Lactococcus lactis*. It is preferably administered with an adjuvant, e.g. alum.

5 The protein may be a mutant protein, in comparison to wild-type protein, a fragment of the protein or a combination of different fragments, provided an effective immune response is generated.

10 An alternative approach is to use a live attenuated GBS vaccine. This may be produced by deleting the gene that encodes the protein. Preferably, the GBS strain comprises additional virulence gene mutations.

The protein (or fragments thereof) of the present invention may also be used to produce monoclonal and polyclonal antibodies for use in passive immunisation.

15 In a further embodiment of the invention, the protein or corresponding polynucleotide may be used as a target for screening potentially useful drugs, especially antimicrobials. Suitable drugs may be selected for their ability to bind to the protein to exert their effects.
20 Assays for screening for suitable drugs and which make use of the protein of the invention will be apparent to those skilled in the art.

25 Although the protein has been described for use in the treatment of patients, veterinary uses of the protein are also considered to be within the scope of the present invention. In particular, the protein or the vaccines may be used in the treatment of chronic mastitis, especially in cows.

30 The present invention is described with reference to Group B Streptococcal strain M732. However, all the GBS strains and many other bacterial strains are likely to include related proteins having amino acid sequence homology with the protein of M732. Organisms likely to contain the proteins include, but are not limited to, *S.*

pneumoniae, *S. pyogenes*, *S. suis*, *S. milleri*, Group C and Group G *Streptococci* and *Enterococci*. Vaccines to each of these may be developed in the same way as described for GBS.

5 Preferably, the proteins that may be useful for the production of vaccines have greater than 40% sequence similarity with the protein of M732. More preferably, the proteins have greater than 60% sequence similarity. Most preferably, the proteins have greater than 80%
10 sequence similarity.

 The protein of the present invention was identified as follows:

 A partial gene library of GBS (strain M732) chromosomal DNA was prepared using the plasmid vectors
15 pFW-*phoA*1, pFW-*phoA*2 and pFW-*phoA*3 (Podbielski, A. et al. 1996. Gene 177:137-147). These plasmids possess a constitutive spectinomycin adenyltransferase antibiotic resistance marker, which confers a high level of spectinomycin resistance and is therefore easily
20 selected. Furthermore, these vectors contain a truncated (leaderless) *Escherichia coli phoA* gene for alkaline phosphatase. The three vectors differ only with respect to the reading frame in which the leaderless *phoA* gene exists, as compared to an upstream in-frame *Bam*HI
25 restriction enzyme site. Because this truncated *E. coli phoA* gene lacks the appropriate leader sequence for export of this enzyme across the bacterial membrane, extracellular alkaline phosphatase activity is absent when these plasmids are propagated in an *E. coli phoA*
30 mutant (e.g. strain DH5 α). The chromogenic alkaline phosphatase substrate, XP (5-Bromo-4-chloro-3-indolyl-phosphate), does not enter intact bacterial cells and therefore only exported or surface associated alkaline phosphatase activity can be detected. When exported or

surface associated alkaline phosphatase activity is present, the chromogenic XP substrate is cleaved to yield a blue pigment and the corresponding bacterial colonies can be identified by their blue colour.

5 Plasmid DNA was digested to completion with *Bam*HI and dephosphorylated using shrimp alkaline phosphatase. GBS genomic DNA was partially digested with *Sau*3AI, size fractionated on a sucrose gradient and fragments <1kb in size were ligated into the prepared pFW-*phoA* vectors. *E.*
 10 *coli* strain DH5 α was chosen as the cloning host since it lacks a functional *phoA* gene. Recombinant plasmids were selected on Luria agar containing 100 μ g/ml of spectinomycin and 40 μ g/ml of the chromogenic XP substrate. *E. coli* transformants harbouring plasmids
 15 containing GBS insert DNA that complements the export signal sequence of the leaderless *phoA* gene were identified by the blue colour of the colonies. Approximately 30000 different recombinant plasmids containing GBS insert DNA were screened in this manner
 20 and 83 recombinant plasmids, which complemented the leaderless *phoA*, were chosen for further study.

From these experiments, one clone was selected containing a plasmid designated pho3-18. This plasmid contained a gene (or part thereof), which complemented
 25 the leaderless *phoA*. Plasmid pho3-18 contained 679 bp of GBS DNA and the nucleotide and deduced amino acid sequences are shown in Figure 1.

A comparison of the amino acid sequence of ORF3-18 was performed and the results are shown in Table 1.

30 As shown in Table 1, homologues to the GBS ORF3-18 gene product can be identified in *Streptococcus pyogenes* and *Streptococcus pneumoniae*. These homologues were identified from genome sequence data and no annotations were available as to the identity of the gene or gene

products. Using these *S. pyogenes* and *S. pneumoniae* homologues to search the public databases showed some similarity to outer surface and membrane spanning proteins. Since the ORF3-18 product complemented the
5 leaderless *phoA* gene, it can be concluded that this protein (or part thereof) would most probably be located extracellularly.

Table 1. Database search results for ORF3-18 (57 amino acids)

Organism	Protein Accession	DNA Accession	Gene Name	% Similarity	% Identity	Alignment Length
<i>S. pyogenes</i>	bp 13438-14706	Contig 271 (rev)	Unknown	54.54	40.00	57
<i>S. pneumoniae</i>	bp 6015-7310	Contig 4164	Unknown	42.10	28.07	57

CLAIMS

1. A protein comprising an amino acid sequence encoded by the polynucleotide defined as ORF3-18 in Figure 1 or a homologue thereof with at least 60% sequence homology.
- 5 2. A protein according to claim 1, obtainable from the Group B streptococcal strain M732.
3. A protein according to claim 1 or claim 2, wherein ORF3-18 comprises the nucleotides 508-678.
4. A protein according to any of claims 1 to 3, for use
10 in a method of therapy.
5. A polynucleotide which encodes a protein according to any preceding claim, its complement, or a fragment thereof.
6. The use of a bacterial protein according to any of claims 1 to 4, in the manufacture of a vaccine to treat
15 bacterial infection.
7. The use according to claim 6, wherein the infection is a Group B streptococcal infection.
8. The use according to claim 6 or claim 7, wherein the infection is a focal infection.
- 20 9. The use according to claim 6 or claim 7, wherein the infection is a urinary tract infection.
10. Use of a product according to any of claims 1 to 5, for screening potential antimicrobial drugs.
11. An antimicrobial drug selected using the products as
25 defined in claim 10.
12. A vaccine comprising a product according to any of claims 1 to 5.
13. A vaccine comprising a microorganism having a virulence gene deletion, wherein the gene codes for a
30 protein according to any of claims 1 to 4.
14. An antibody raised against a protein according to any of claims 1 to 4.

Figure 1. Nucleotide and deduced amino acid
sequence of clone pho3-18

```

      10              30              50
GATCAAGTGATGGTTTTTGGTGATGGGGGCAATGATGTGGAAATGTTGAG

      70              90
AGCAGCAAATATTTCTTACGCTATGTCAAATGCGCCAGAAGAAATTAAAG

     110             130             150
CTATTGCTAAATATCAAACAGTTAGTAACGATCGCTACATTAATCTTTAC

     170             190
CAAGCGATTGGAATGCCAACTACACTTGCAGAACTCCATTTGGGTGATGC

     210             230             250
AACATATGAAGAACCTTTTAAAAGTTGGTCAGCAAGCAACCAATTGAAGG

     270             290
TGAAACAATACCATGAAATGCCATTCAAAATTAGTGCAGAGGATGTCGCT

     310             330             350
GCAGCCCTGTTAGCAGTTGACCGCTATGTTTCAAATCATCAATAAAAACA

     370             390
ATAGAACCAAGTGAAATTGCTTGGGTTCTATTTGTCGTTTTAATATTTTA

     410             430             450
ACTAAATAGGGCTTACTAGTATTTAAAGAGGATATAAACTAAAAAAGATC

     470             490
CTATGAGTAACAGTGAGCGAAAACTGTTCATAAAACAATTGCAACTATA
      START ORF3-18
      |
     510             530             550
GAGGGTGTTGAAAGTTATTCTGAAGGTAACGATCATTGTACTAGCTAGTC
      L K V I L K V T I I V L A S L

     570             590
TGACAGTGACGATAATTTCTTACCCAAAAATGACGGAATTAACAAAGTCC
      T V T I I S Y P K M T E L T K S

     610             630             650
GTTGAAAAACAACCTTGAAGATAATGCTGATAATCTATCAGACCAACTGAC
V E K Q L E D N A D N L S D Q L T

     670
ATATCAGATAGAAGTGGCGCAAAAAGATC
      Y Q I E V A Q K D

```

PCT/G B 99/04377
23/77 Ad 22-12-99

Gill Jennings + Every

THIS PAGE BLANK (USPTO)